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SHORT REPORT

Association of antiphospholipid antibodies with active digital ulceration in systemic sclerosis

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Raynaud's phenomenon (RP) in systemic sclerosis (SSc) can be severe with active digital ulceration (ADU) and gangrene. RP physiopathology includes early endothelial cell injury, vascular dysfunction and sometimes microvascular thrombosis.[1] Antiphospholipid antibodies (aPL) activate endothelial cells and platelets by complexes of beta-2 glycoprotein 1 (β2GP1) and anti-β2GP1[2] and could therefore contribute to the initiation of and/or aggravate SSc-related RP. aPL prevalence seems increased in patients with SSc compared with controls,[3] but aPL-associated clinical features are often contradictory. The aims of this study were to assess aPL and antiphospholipid syndrome (APS) prevalence in patients with SSc and their association with ADU.

Consecutive patients aged more than 18 years, fulfilling the American College of Rheumatology/EULAR classification criteria for SSc[4] and followed in seven French expert centres for autoimmune diseases labelled by the French national network for autoimmune disease care, were enrolled prospectively during 8 months. Patients with other associated autoimmune disease were excluded. All patients provided written informed consent. SSc subtype was classified based on LeRoy and Medsger's criteria,[5] and skin involvement was assessed according to the modified Rodnan skin score (mRSS).[6] Interstitial lung disease (ILD) was defined by subpleural ground-glass opacities and/or interstitial reticular pattern with or without fibrosis on high-resolution CT. Raynaud's activity and the presence of digital ischaemia was determined using a previously published severity score: 0—no Raynaud's; 1—Raynaud's with/without vasodilator required; 2—Digital Pitting Scars; 3—Digital Tip Ulcerations and 4—Digital Gangrene.[7] ADU was defined as a score ≥3.[8] Pulmonary arterial hypertension (PAH) was defined by median pulmonary arterial pressure (mPAP) ≥25 mm Hg and pulmonary arterial wedge pressure ≤15 mm Hg, not related to lung diseases and/or chronic thromboembolism. Screening for lupus anticoagulant (LA), anticardiolipin (aCL) and anti-β2GP1 antibodies was systematically carried out in each centre and confirmed at least 12 weeks apart. Only persistent aPL were considered.[9] LA was detected according to the international recommendations.[10] Positivity for immunoglobulin (Ig)G/IgM aCL and anti-β2GP1 in immunosorbent assay was defined as 'overall' (titres more than 15–20 U/mL, according to each manufacturer's guidelines) and 'high' (titres >40 U/mL[9]). APS was diagnosed according to international definition.[9] Statistical analysis was performed using SAS V.9.4.

Key messages

What is already known about this subject?

- Antiphospholipid antibodies (aPL) prevalence seemed increased in patients with systemic sclerosis (SSc), but their clinical signification is unclear.

What does this study add?

- Description of aPL positivity and antiphospholipid syndrome prevalence in a multicentre cross-sectional SSc cohort (confirmed and high aPL titre level).
- Anti-beta-2 glycoprotein 1 (β2GP1) positivity is an independent factor associated with active digital ulceration.

How might this impact on clinical practice?

- aPL and especially anti-β2GP1 should be screened in SSc to detect patients potentially at higher risk of developing digital ulcers.

Comparison for scaled variables were performed using Student's t-test. Association between qualitative variables was determined with Pearson's χ^2 or Fisher's exact tests. Factors independently associated with ADU were studied by logistic regression model. Dependent variables were selected using a manual backward procedure with p=0.05 as threshold for exit. The procedure initially included all factors associated with severe RP with a p-value below 0.1 in bivariate analysis. Subanalyses were performed considering only 'high' titres of aCL and/or anti-β2GP1.[9] The alpha risk was set at 5% and statistical tests were performed bilaterally with p<0.05 considered as significant. Data are available upon request.

One hundred and sixty-eight patients were included. Their characteristics are summarised in table 1. LA was detected in 17 (10%) patients. Overall, aCL and/or anti-β2GP1 were present in 14 (8%) and 14 (8%) patients, respectively. Positivity for ≥1 aPL was found in 31 (18%) patients and triple positivity in three (2%). Seven (4%) patients had definite APS, without obstetric APS. Anti-Scl70 positivity was more frequent in the APS group (71% vs 34%, p=0.046). No association was observed between APS and other SSc-related manifestations, including PAH (online supplementary table 1). Among the 31 aPL-positive patients, 12 had ADU among whom all received calcium channel inhibitors, seven had bosentan, nine had low-dose aspirin while three received vitamin K antagonists or unfractioned heparin for APS (online supplementary table 2). Forty-eight patients (28.6%) had ADU (table 2). In univariate analysis, factors associated with ADU were positivity for anti-β2GP1 IgG (p<0.001), anti-Scl70 (p=0.006) and higher mean mRSS (6.0 vs 14.0, p<0.001). Anti-β2GP1 positivity and mRSS remained significant in multivariate analysis (OR 8.71, 95% CI 1.31 to 55.43; p=0.02 and OR 1.06, 95% CI 1.02 to 1.11; p=0.007, respectively). When considering only 'high' aPL titres, anti-β2GP1 and anti-β2GP1 IgG remained significant (p=0.003 both) in univariate analysis, and anti-β2GP1 IgM was also associated with ADU (p=0.04). In multivariate analysis, only mRSS remained significantly associated with ADU (OR 1.07, 95% CI 1.02 to 1.11; p=0.005) with a trend towards anti-β2GP1 positivity (OR 5.27, 95% CI 0.97 to 28.62; p=0.054) (online supplementary table 3) (online supplementary table 3). aPL, APS and past thrombotic event prevalences according to the different RP subsets are depicted in online supplementary table 4.

Overall, aPL prevalence in our population (18%) is consistent with those recently reported in worldwide (14%) and European (15%) SSc populations,[11] despite pronounced heterogeneity between studies in terms of aPL definition and technical methodology. We report an estimated APS prevalence in SSc of 4%. Notwithstanding its rare occurrence, the association between APS and anti-Scl70 merits further evaluation.

Overall, anti-β2GP1 positivity was independently associated with ADU. A recent metanalysis did not find any association between overall aPL, LA, aCL or anti-β2GP1

Table 1 Patient characteristics

Characteristics		Patients (n=168)
Age, years±SD		57.8±15
Female, n (%)		147 (87.5)
Smoking, n (%)		
Current or past		20 (12)
Never		136 (81)
NA		12 (7)
SSc type, n (%)		
Limited		69 (41)
Diffuse		99 (59)
Disease duration, years±SD*		8.2±13.5
Modified Rodnan skin score (0–51), mean (Q1, Q3)		8.0 (4.0, 19.0)
ADU, n (%)		48 (29)
Digital tip ulcerations, n (%)		44 (26)
Digital gangrene, n (%)		4 (3)
Raynaud's severity score (0–4), mean (Q1, Q3)		1.0 (1.0, 3.0)
PAH		
Suspected PAH on echocardiography, n (%)		19 (11)
sPAP (mm Hg), mean±SD		31.8±13.7
PAH on RHC, n (%)		12 (7)
mPAP (mm Hg), mean±SD		30.9±15
LVEF on echocardiography (%), mean±SD		66±9
ILD, n (%)		73 (43)
FVC, % predicted ±SD		94.6±24.7
DLCO, % predicted ±SD		69.1±21.1
SSc-related autoantibodies, n (%)		
Anticentromere, n (%)		76 (45)
Anti-Scl70, n (%)		60 (36)
LA, n (%)		17 (10)
	Overall	**High (>40 U/mL)**
aCL, n (%)		
Any isotype (global)	14 (8)	6 (4)
IgG	11 (6)	6 (4)
IgM	3 (2)	0 (0)
Anti-β2GP1, n (%)		
Any isotype	14 (8)	10 (6)
IgG	8 (5)	6 (4)
IgM	8 (5)	4 (3)
≥1 aPL, n (%)	31 (18)	26 (15)
Triple positivity, n (%)	3 (2)	1 (1)
APS, n (%)	7 (4)	
Related-arterial thrombosis, n (%)	4 (2)	
Related-venous thrombosis, n (%)	3 (2)	
Obstetrical manifestation, n (%)	0 (0)	
Overall arterial thrombosis history, n (%)	5 (3)	
Overall venous thrombosis history, n (%)	13 (8)	

*Time from first non-Raynaud's symptom.
aCL, anticardiolipin; ADU, active digital ulceration; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; β2GP1, beta-2 glycoprotein 1;Ig, immunoglobulin; ILD, interstitial lung disease; LA, lupus anticoagulant; LVEF, left ventricular ejection fraction; mPAP, mean pulmonary arterial pressure;NA, not available; PAH, pulmonary arterial hypertension; Q1/Q3, quartile 1/quartile 3; RHC, right heart catheterisation; sPAP, systolic pulmonary arterial pressure; SSc, systemic sclerosis.

Table 2 Factors associated with ADU

Characteristics	ADU– (n=120)	ADU+ (n=48)	P value	Adjusted OR (95% CI)*
Age, years±SD	58.9±15.5	55.2±13.2	0.15	ND
Female, n (%)	107 (89.2)	40 (83.3)	0.31	ND
Smoking, n (%)			0.43	ND
Current or past	13 (11)	7 (15)		
Never	100 (83)	36 (75)		
NA	7 (6)	5 (10)		
SSc type, n (%)			0.80	ND
Limited	50 (42)	19 (40)		
Diffuse	70 (58)	29 (60)		
Disease duration, years±SD†	7.3±12.7	10.4±15.1	0.19	ND
Modified Rodnan skin score (0–51), mean (Q1, Q3)	6.0 (2.0, 14.5)	14.0 (8.0, 26.0)	**<0.001**	**1.06 (1.02 to 1.11) (p=0.007)**
PAH				
Suspected PAH on echocardiography, n (%)	13 (11)	6 (13)	0.76	ND
sPAP (mm Hg), mean±SD	31.9±14.7	31.5±10.9	0.90	
PAH on RHC, n (%)	8 (7)	4 (8)	0.70	
mPAP (mm Hg), mean±SD	31.8±9.6	29.6±21.8	0.73	
LVEF on echocardiography (%), mean±SD	65.4±9.8	67.6±6.7	0.23	ND
ILD, n (%)	47 (39)	26 (54)	0.08	ND
FVC, % predicted ±SD	97.3±17.4	88.3±26.9	0.09	ND
DLCO, % predicted ±SD	71±21.2	64.8±20.4	0.14	ND
SSc-related autoantibodies, n (%)				
Anticentromere, n (%)	60 (50)	16 (33)	0.05	0.64 (0.2 to 1.87)
Anti-Scl70, n (%)	35 (29)	25 (52)	**0.006**	2.55 (0.91 to 7.14)
aPL, n (%)				
LA	10 (8)	7 (15)	0.22	ND
aCL (overall positivity)	11 (9)	3 (6)	0.54	ND
IgG	8 (7)	2 (4)	0.57	ND
IgM	2 (2)	1 (2)	0.83	ND
Anti-β2GP1 (overall positivity)	5 (4)	9 (19)	**0.002**	**8.71 (1.31 to 55.43) (p=0.02)**
IgG	1 (1)	7 (16)	**<0.001**	ND
IgM	4 (4)	4 (9)	0.17	ND
APS, n (%)	4 (3)	3 (6)	0.39	ND
Overall arterial thrombosis history, n (%)	3 (3)	2 (4)	0.61	ND
Overall venous thrombosis history, n (%)	12 (10)	1 (2)	0.08	ND

*Logistic regression with adjustment for anticentromere, anti-Scl70, modified Rodnan skin score, smoking, the presence of LA and/or aCL.
†Time from first non-Raynaud's symptom.
aCL, anticardiolipin; ADU, active digital ulceration; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; β2GP1, beta-2 glycoprotein 1;Ig, immunoglobulin; IPD, interstitial pulmonary disease; LA, lupus anticoagulant; LVEF, left ventricular ejection fraction; mPAP, mean pulmonary arterial pressure; NA, not available ;ND, not done; PAH, pulmonary arterial hypertension; Q1/Q3, quartile 1/quartile 3; RHC, right heart catheterisation; sPAP, systolic pulmonary arterial pressure; SSc, systemic sclerosis.

positivity and digital ulcers.[11] Only three studies have specifically evaluated IgG and/or IgM aCL prevalence in patients with SSc with or without digital ulcer, and no difference was observed.[3] Only one study has shown an independent association between active digital ischaemia and IgM anti-β2GP1 positivity, using the same definition of ADU as us.[8] On the other hand, another study reported an association between aCL positivity and elevated von Willebrand antigen factor.[12] Taken together, these results suggest possible associations between aPL positivity and endothelial injury in SSc, and this relationship deserves further evaluation.

The main strength of our study was to only consider confirmed aPL and subanalyses with high titres of aPL, thereby avoiding potential bias of transient and non-clinically relevant aPL. Moreover, distinction of each aPL type and isotype was made for assessment of potential associations with ADU. Lastly, our results reflect a real-life prevalence study of aPL in SSc with aPL assays having been performed in each centre.

Our study has several limitations. First, the lack of power precluded multivariate analysis for aCL and anti-β2GP1 isotypes and probably explains the loss of significance for the association between 'high' anti-β2GP1 positivity

and ADU. Moreover, the low number of patients with APS calls for cautious interpretation of the data. Second, thrombosis history was collected retrospectively, leading to potential memorisation and selection bias.

In conclusion, this study found an aPL prevalence in SSc of 18%. Anti-β2GP1 positivity is an independent factor associated with ADU and might be preferentially related to positivity of antiB2GP1 IgG isotype. These data provide additional insights into the role of aPL in SSc vasculopathy.

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